Exercise-induced increases in cardiac sarcoplasmic reticulum calcium-ATPase 2 (SERCA2) expression and activity is attenuated in AMPKα2 kinase dead mice

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Exercise-induced increases in cardiac sarcoplasmic reticulum calcium-ATPase 2 (SERCA2) expression and activity is attenuated in AMPKα2 kinase dead mice

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Abstract

Exercise enhances cardiac sarcoplasmic reticulum Ca\(^{2+}\)-ATPase 2a (SERCA2a) function through unknown mechanisms. The present study tested the hypothesis that the positive effects of exercise (Ex) on SERCA2a expression and function in the left ventricle (LV) is dependent on adenosine monophosphate-activated protein kinase (AMPK) \(\alpha_2\) function. AMPK\(\alpha_2\)-kinase dead (KD) transgenic mice, which overexpress inactivated AMPK\(\alpha_2\) subunit, and wild-type C57Bl/6 (WT) mice were randomized into sedentary groups or groups with access to running wheels. After 5 months, exercised KD mice exhibited shortened deceleration time compared to sedentary KD mice. In LV tissue, the ratio of phosphorylated AMPK\(\alpha_{\text{Thr}172}\):total AMPK\(\alpha\) was 65% lower (P<0.05) in KD mice compared to WT mice. The LV of KD mice had 37% lower levels of SERCA2a compared to WT mice. Although exercise increased SERCA2a protein levels in WT mice by 53%, this response of exercise was abolished in exercised KD mice. Exercise training reduced total phospholamban (PLN) protein content by 23% in both the WT and KD mice but remained 20% higher overall in KD mice. Collectively, these data suggest that AMPK\(\alpha\) influences SERCA2a and PLN protein content in the sedentary and exercised heart, and that exercise-induced changes in SERCA2a protein are dependent on AMPK\(\alpha\) function.

Key Words: AMPK; SERCA; exercise training; phospholamban; \(\text{Ca}^{2+}\) handling, left ventricle; diastolic function
**Introduction**

Physical activity is an important factor for the prevention of heart disease and also an important component of rehabilitation for patients with established cardiovascular disease (Perez-Terzic 2012). Even so, the molecular mechanisms by which exercise therapy improves heart health have yet to be identified. Wisloff et al. (Wisloff et al. 2002) previously reported that exercise training started 4 weeks after ligation of the left anterior descending artery in rats restores contractile function and myocardial calcium-handling in failing hearts. More recently, Ait et al. (Ait et al. 2009) demonstrated that exercise training started thirteen weeks post-myocardial infarction in rats can partially restore sarcoplasmic reticulum calcium-ATPase (SERCA2a) protein content and cardiac function in rats. Exercise training also enhances myocardial calcium-cycling by improving SERCA2a function in the diabetic heart (Stolen et al. 2009). Our previous work indicated that exercise training prevents the dysregulation of myocardial SERCA2a protein content in the low-dose streptozotocin, high fat fed rat model (Epp et al. 2013). However, a major limitation of the existing literature is the failure to identify the underlying mechanisms by which exercise training enhances SERCA2a protein content and function in the heart.

SERCA2a mRNA (Arai et al. 1993; Mercadier et al. 1990; Schwinger et al. 1999) and activity (Arai et al. 1993; Mercadier et al. 1990; Schwinger et al. 1999) were lowered in patients with heart failure; while SERCA2a protein and activity were reduced in models of diabetes (Stolen et al. 2009) and aging (Turdi et al. 2010). Similarly, aging decreases the activity of adenosine monophosphate-activated protein kinase (AMPK), which is an energy sensor in cardiac and skeletal muscle (Shirwany and Zou 2010), as it senses increases in adenosine monophosphate (AMP) levels following exercise or other metabolic challenges (Li and Keaney 2010). AMPK is
a heterotrimeric serine/threonine kinase and is composed of an α-catalytic subunit and noncatalytic β- and γ-subunits (Richter and Ruderman 2009). Two isoforms of the AMPKα catalytic subunit, AMPKα₁ and AMPKα₂, are co-expressed in cardiac and skeletal muscle (Stapleton et al. 1996). AMPK stimulates energy metabolism and the transition from anabolic to catabolic processes (Steinberg and Kemp 2009). For example, although exercise does not impact AMPKα₁ activity, AMPKα₂ activity increases 2.7-fold during an acute bout of exercise and remains activated for at least 30 minutes post-exercise in human skeletal muscle biopsied from humans with or without diabetes (Musi et al. 2001).

Studies that have utilized either muscle specific AMPKα₂ kinase dead transgenic mice, which is an animal model where cardiac and skeletal muscle AMPKα₂ activity was found to be negligible (Russell et al. 2004), or whole-body AMPKα₂ knockout mice have supported the model that the AMPKα₂ isoform is essential for contraction-stimulated metabolic changes in cardiac (Habets et al. 2009) as well as skeletal muscle (Jorgensen et al. 2007; Lefort et al. 2008). There is also evidence indicating that AMPK influences pathways that influence cellular energy utilization. For example, AMPK influences exercise-induced fiber type transition (i.e. myosin heavy chain IIb to IIa/x) in skeletal muscle (Rockl et al. 2007). AMPK signaling also influences SERCA protein content and function in endothelial cells (Dong et al. 2010) and in the heart (Turdi et al. 2010). In fact, Turdi et al. (Turdi et al. 2010) have reported that myocardial SERCA2a protein levels are compromised with aging and that this age-dependent reduction was even greater in hearts isolated from AMPKα₂ kinase dead transgenic mice. An AMPKα-mediated process also appears to influence the exercise training induced changes in SERCA1a and SERCA2a isoform expression in skeletal muscle (Morissette et al. 2014). However, the role
of AMPKα in the regulation of myocardial SERCA2a protein content following exercise training is unknown.

The present study tested the hypothesis that SERCA2a expression and function measured in the left ventricle (LV) of AMPKα2-kinase dead (KD) transgenic mice will not be enhanced by exercise (Ex) training, as compared to sedentary (Sed) KD mice; whereas, Ex will increase SERCA2a expression and function in wild-type (WT) mice.

Materials and methods

The Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines were followed in the development of this manuscript (Kilkenny et al. 2010).

Animal model

Animals were treated in accordance with the guidelines of the Canadian Council on Animal Care (2012) and the experimental protocol (No. 10-056) was approved by the University of Manitoba Animal Protocol Management and Review Committee. Two male KD mice were provided to us by Dr. Morris Birnbaum (University of Pennsylvania) from the Tg-KD1 line (Mu et al. 2001). These KD mice were then used to establish a breeding colony with C57Bl/6 mice in our own conventional mouse housing facility. The heterozygous transgenic mice overexpress a dominant kinase dead AMPKα2 protein isoform whose expression is driven by a muscle-specific creatine kinase (MCK) promoter, resulting in the inactivation of the α2 catalytic subunit in cardiac and skeletal muscle (Mu et al. 2001). Others have reported that this transgenic manipulation reduced cardiac AMPKα2 activity from 50-100%, while the activity of AMPKα1...
was reduced by up to 46% (Habets et al. 2009; Russell et al. 2004; Turdi et al. 2011). Mice were genotyped using tail-snip samples and PCR, using forward primer (5’-CGAGGTCGACGTTACGATAAGCTTGATATC-3’) and reverse primer (5’-GAAGGAACCCGTTGGAGGACTGGAGGCGAGG-3’).

Animals from each genotype were allocated to each of the sedentary or exercise groups randomly, generating four groups: (1) non-exercise trained, sedentary (Sed) AMPKα2 KD mice (n=4 male mice, n=4 female mice; 8 weeks old) (KD+Sed), (2) non-exercise trained, sedentary (Sed) wild-type (WT, C57Bl/6) (n=4 male mice, n=4 female mice; 8 weeks old) (WT+Sed), (3) exercise trained (Ex) AMPKα2 KD mice (n=4 male mice, n=4 female mice; 8 weeks old) (KD+Ex), (4) exercise trained (Ex) wild-type (WT, C57Bl/6) (n=4 male mice, n=4 female mice; 8 weeks old) (WT+Ex).

All mice were fed a standard diet (Test Diet product 5TJS; 12% kcal from fat, 72% kcal from carbohydrates and 16% kcal from protein) for 5 months ad libitum with free access to drinking water. Control and experimental mice were housed in cages (1 mouse/cage) containing wood chip bedding (Sani-Chip, P.J. Murphy Forest Products Corp.) on a 12:12-h light-dark cycle. Cage level assessments occurred daily to ensure health of mice. Our decision to use both sexes was twofold. First, we wanted to reduce the number of animals that had to be bred for this research, which is consistent with the Canadian Council on Animal Care Guidelines. Second, we made the assumption that there was no sex difference in the regulation of Ca²⁺-handling; however, recent data challenges this assumption because cardiomyocytes isolated from female mice have smaller contractions and Ca²⁺-transients than males due to lower cAMP levels caused by higher
expression of phosphodiesterase (PDE) 4B (Parks et al. 2014). This sex-specific effect appears to reduce ryanodine receptor calcium release in cardiomyocytes isolated from female mice despite similar sarcoplasmic reticulum Ca$^{2+}$-content and diastolic Ca$^{2+}$ levels between sexes. However, it remains unknown if sex differences influence the regulation of SERCA2a in the heart. Data from 4 male and 4 female mice from each group were used to generate exercise training, cardiovascular imaging and qPCR data. SERCA2a activity was used as the primary outcome variable. A sample size calculation indicates that an n of 7 per group will be needed to detect differences between groups (two tailed $\alpha=0.05; \beta=0.80$; Mean ± SD, Group 1, 104 ± 21; Group 2, 71 ± 17). We are mindful that our sample may have higher variability than that used in the calculation, so we have adjusted the sample size to an n of 8 for the primary outcome variable. The sample size used in the current study was not powered to compare differences between males and females.

The general characteristic of the animals used in this study have been described in a previous publication by our group (Morissette et al. 2014). To determine whether exercise training altered running capacity, a graded exercise treadmill test was performed at the end of the study protocol.

**Exercise training and graded exercise testing**

Wheel running has been used previously to enhance AMPK activity *in vivo* (Leick et al. 2008; Thomson et al. 2007). Exercise trained mice were housed in cages with voluntary running wheels for the duration of the 5-month protocol (Ex) while non-exercise trained, sedentary mice were housed in standard cages (Sed).
Exercise-stimulated changes in mouse running capacity were measured using a modified graded treadmill exercise test previously described by Hoydal et al. (Hoydal et al. 2007). Maximal running speed achieved during this graded exercise test is correlated with peak aerobic fitness ($VO_{2\text{max}}$) (Hoydal et al. 2007). For baseline testing, mice started running on a Columbus Instruments Exer-3/6 Treadmill at a speed of 7 m/min with the speed increased by 1.6 m/min until fatigue, which was defined as the speed at which animals could no longer maintain the specific running pace. For follow-up testing, mice were warmed up at 60% of their previous max speed for 2 minutes, followed by 2-minute intervals at 70%, 80% and 95% of their previous max speed. From this point, the treadmill speed was increased by 0.8 m/min until the animal was fatigued. Graded exercise tests were conducted monthly (i.e. baseline, 1, 2, 3, 4 and 5 months); however, only the 5-month data have been reported (n=8 for each of the sedentary or exercised trained groups).

**Echocardiography**

Echocardiography was utilized to characterize changes in left ventricular structure and function \textit{in vivo}, as previously described (Jassal et al. 2009a; Jassal et al. 2009b; Syed et al. 2005). Briefly, non-invasive echocardiography was performed using both a 13-MHz probe (Vivid 7, GE Medical Systems) and a 40 MHz probe (Vevo 2100, Visualsonics) at the completion of the 5-month study (n=8 for each of the sedentary or exercised trained groups). Hearts were imaged in the 2-dimensional parasternal short axis view to enable the measurement of left ventricular (LV) internal dimension measured at the end of diastole (LVID$_d$) and left ventricular posterior wall thickness at diastole (LVPW$_d$). End-systolic and end-diastolic volumes were measured from a parasternal long-axis view using the prolate-ellipsoid geometric model, which enabled the
calculation of LV ejection fraction (LVEF). Tissue Doppler imaging was also acquired in the parasternal short axis view at the level of the papillary muscles of the LV, where endocardial velocity was measured on the posterior wall to determine whether systolic dysfunction was present. Diastolic variables measured from the parasternal long-axis view included the peak early (E wave) and late (A wave) diastolic filling velocity, E/A ratio, and deceleration time (DT) of early filling of mitral inflow.

**Tissue isolation**

Upon completion of the 5-month exercise training protocol, animals were removed from the running wheel cages approximately 2 hours before tissue collection. Mice were anesthetized by intraperitoneal injection of ketamine-xylazine (150:100 mg·kg⁻¹) in accordance with the guidelines of the Canadian Council on Animal Care (2012) and the regulations and policies of the University of Manitoba Animal Protocol Management and Review Committee. The LV was rapidly removed and sliced longitudinally. One of these sections was frozen by flash freezing in liquid nitrogen for later analysis while the remainder of the LV was immediately diluted 1:10 (w/v) and homogenized in ice-cold buffer (pH 7.5) containing 250 mM sucrose, 5 mM HEPES, 0.2 mM phenylmethylsulfonyl fluoride (PMSF) and 0.2% sodium azide (NaN₃). The tissues were homogenized using an all glass tissue grinder (Kimble Chase Glassware). The data reported reflects the condition of the left ventricle following anesthesia, tissue preparation and analytical procedures. We did not utilize β-blockers or Ca²⁺/calmodulin-dependent protein kinase (CaMKII)-blockers or inhibitors other than those indicated above in the preparation of tissue samples. Tissue homogenates were stored at -80°C until needed for analyses. Total protein content was quantified in triplicate for each sample using the bicinchoninic acid (BCA) protein.
assay and in preparation for Western analysis, 20 μg of protein sample was denatured by the
addition of Laemmli buffer. Samples were heated to 90°C for 5 min. To assess PLN pentamer:
monomer ratios, purified SERCA2a vesicles were prepared and assessed using a PLN antibody
by comparing the 30 kDa band in non-boiled samples (which represent PLN pentamers) to the 5
kDa band in samples that were boiled for 10 min (which represents total PLN monomers).
Samples were boiled to disrupt PLN pentamer interactions, thereby causing PLN to exist in the
monomeric form.

The mice utilized in this investigation to explore heart tissue were the same mice as those used
to explore skeletal muscle in a previous study (Morissette et al. 2014), and all tissue was
analyzed shortly after collection.

**Immunoblotting**

Tissues from 3 male and 2 female mice (n=5) per group (N=20) were analyzed by western
blotting. Twenty micrograms of LV tissue homogenate were loaded in each well and resolved on
7.5, 10, 12.5 and 15% SDS-polyacrylamide gels, followed by semi-dry transfer onto
polyvinylidene difluoride membranes (Bio-Rad Laboratories). Following blocking (5% w/v BSA
in tris-buffered saline with 0.1% Tween-20) for 1 h at room temperature, blots were immuno-
labeled at 1:1000 with anti-SERCA2a (No. 4388, Cell Signaling Technology), anti-AMPKα (No.
2532, Cell Signaling Technology), anti-phosphorylated-AMPKα<sup>Thr172</sup> (p-AMPKα<sup>Thr172</sup>; No.
4188, Cell Signaling Technology), anti-PLN (PLN; No. sc-21923, Santa Cruz Biotechnology,
Inc.), anti-phosphorylated-PLN<sup>Thr17</sup> (p-PLN<sup>Thr17</sup>; No. sc-17024-R, Santa Cruz Biotechnology,
Inc.), anti-phosphorylated-PLN<sup>Ser16</sup> (p-PLN<sup>Ser16</sup>; No. sc-12963, Santa Cruz Biotechnology, Inc.),
anti-acetyl-CoA carboxylase (ACC; No. 3662; Cell Signaling Technology), anti-phosphorylated-ACC (No. 3661; Cell Signaling Technology) and anti-cytochrome C oxidase (COX IV; No. 4844, Cell Signaling Technology) primary antibodies and appropriate horseradish peroxidase-conjugated secondary antibodies. Blots were then visualized using ECL reagent (No. 32106, Thermo Scientific or No. RPN2232, Amersham) for 5 min and images detected with a Fluor-S-Max MultiImager (Bio-Rad Laboratories). Anti-β-tubulin antibody (No. 2128, Cell Signaling Technology) was used as gel loading control. Relative protein levels were first expressed as a percentage of the corresponding β-tubulin level, and then expressed as a percentage of control.

RNA isolation and real-time polymerase chain reaction (RT-PCR)

Total RNA was harvested from LV tissue (n=8 for each of the sedentary or exercised trained groups) using TRIzol reagent (Invitrogen) according to manufacturer’s protocol. Total RNA (200 ng) first underwent a DNase digestion (Invitrogen) as per manufacturer’s protocol followed by amplification using a one-step real-time RT-PCR kit (Bio-Rad Laboratories) on an IQ5 RT-PCR system (Bio-Rad Laboratories) with the following primer sets: SERCA2 (F) 5’-TGGAGAACGCTCACACAAAG-3’, (R) 5’-CTCAATCACAAGTTCCAGCA-3’; PLN (F) 5’-CTTTTGCTTTCTGGCATAA-3’, (R) 5’-AGGTTCTGGAGATTCTGACG-3’; GAPDH (F) 5’-TGCACCACAACTGCTTAGC-3’, (R) 5’-GGCATGGACTGTGGTCATGAG-3’. GAPDH was used as a control to account for any variations in the amount of RNA input and the efficiency of reverse transcription.

Assessment of SERCA ATPase activity
Measurements of the kinetic properties of Ca\(^{2+}\)-dependent SERCA activity in LV tissue homogenates were completed using a spectrophotometric assay (Simonides and van Hardeveld 1990) in the presence of 1 \(\mu\)M Ca\(^{2+}\)-ionophore A23187, as modified by Duhamel et al. (Duhamel et al. 2007) for use on a plate reader (SPECTRAmax; Molecular Devices). This protocol enables the analysis of 3 different samples simultaneously on a single plate at 37°C. Cyclopiazonic acid (40 \(\mu\)M), which is a specific inhibitor of SERCA, was included in one reaction for each sample. SERCA-dependent ATPase activity was then calculated based on the difference between the ATP hydrolysis rate stimulated by Ca\(^{2+}\) in the absence and presence of cyclopiazonic acid (Seidler et al. 1989). Kinetic parameters analyzed included maximal SERCA activity (\(V_{\text{max}}\)), Hill coefficient (\(n_H\)), and calcium sensitivity (Ca\(_{50}\); \(n=8\) for each of the sedentary or exercised trained groups).

Statistical analyses

A two-way ANOVA was utilized to detect differences between animal groups based on genotype or training status or for an interaction of genotype and training status. When significant differences (\(p<0.05\)) were detected, a Newman-Keuls post-hoc test was used to identify differences between specific means. Data is presented as mean ± SEM.

Results

General characteristics of animals

Over the full duration of the 5-month protocol, exercise-trained WT+Ex and KD+Ex mice that had access to voluntary running wheels ran an average of 4 ± 1 km/day and 5 ± 1 km/day, respectively. A main effect of genotype was observed for maximum speed achieved during the
graded exercise treadmill test, in which maximal running speed achieved by the KD mice was 18% slower than WT mice. Furthermore, exercise trained mice achieved a 44% faster maximal running speed than their sedentary counterparts (P < 0.01) (Morissette et al. 2014).

Exercise training improves diastolic function in AMPKαKD mice

In vivo echocardiography was utilized to characterize parameters of cardiac structure and function (Table 1). M-mode echocardiography indicated that LVIDd was 13% larger in sedentary KD animals, as compared to sedentary WT animals. Exercise training stimulated a 13% increase in LVIDd in WT+Ex mice, as compared to WT+Sed mice. However, exercise training did not alter LVIDd in KD+Ex mice. No differences in LVPWd, LVEF, endocardial velocity, E wave, A wave, or E/A ratio were observed between any groups. DT was 10.5 ms longer amongst KD+Sed mice, as compared to WT+Sed mice. Exercise training did not alter DT amongst WT+Ex mice, but it did reduce DT by 13.3 ms amongst KD+Ex, as compared to KD+Sed mice. A main effect of exercise training was observed for resting heart rate, where exercise trained mice had heart rates that were significantly lower (5%, P < 0.05) than their sedentary counterparts.

Exercise training enhanced total AMPKα protein and phosphorylation levels in WT but not KD mice

We investigated the protein levels of both the total and phosphorylated forms of AMPKα in LV tissue. Genotype did not alter total AMPKα protein content (Figure 1A). However, total AMPKα protein content was increased by 41% in exercise trained mice, as compared to WT+Sed and KD+Sed mice (Figure 1A). Next, we examined the phosphorylation status of AMPK in order to determine whether the kinase was active. The amount of p-AMPKαThr172 was
65% lower in KD+Sed mice, as compared to WT+Sed mice (Figure 1B). Exercise training enhanced the level of p-AMPKα<sup>Thr172</sup> by 63% in WT+Ex mice, as compared to WT+Sed mice (Figure 1B). However, exercise training did not alter p-AMPKα<sup>Thr172</sup> amongst KD mice. The p-AMPKα<sup>Thr172</sup>:AMPKα ratio was 65% lower for both KD+Sed and KD+Ex mice as compared to their WT counterparts (Figure 1C). Next, we characterized the levels of total and phosphorylated forms of ACC because it is a downstream target of AMPKα. Total ACC levels were not different amongst the different groups (Figure 1D). However, p-ACC levels were 27% lower levels in KD mice than in their WT counterparts (Figure 1E). A main effect of exercise was observed for p-ACC:ACC ratio, where WT+Sed and KD+Sed mice had 64% lower ratios than their exercise trained counterparts (Figure 1F). However, no interaction of genotype and exercise training were detected. We assessed COX IV protein content to determine if genotype or exercise training altered mitochondrial oxidative capacity. Genotype did not alter COX IV protein content (Figure 1G). However, exercise training increased COX IV protein levels by 79% as compared to WT+Sed and KD+Sed mice.

**KD mice were characterized by lower levels of SERCA2 protein**

SERCA2 mRNA expression was altered by genotype in that KD mice had 32% lower mRNA expression than their WT counterparts (Figure 2A). Likewise, KD+Sed mice expressed 37% less SERCA2a protein content than their WT+Sed counterparts (Figure 2B). Although exercise training had no effect on SERCA2 mRNA expression in either WT+Ex or KD+Ex mice compared to sedentary mice (Figure 2A), SERCA2a protein expression was increased by 53% in WT+Ex mice, as compared to WT+Sed mice (Figure 2B). In contrast, exercise training did not alter the content of SERCA2a protein in LV samples isolated from KD+Ex mice. In fact,
SERCA2a protein content was 60% lower in KD+Ex mice, as compared to WT+Ex mice. Next, we assessed the kinetic properties of calcium-dependent SERCA activity using a spectrophotometric assay. KD mice had 17% lower maximal SERCA activity (V_{max}) than the WT mice (Figure 2C). Similarly, Hill coefficient (\eta_H), which quantifies the interaction between SERCA activity and cytosolic free Ca^{2+} for 10-90% V_{max}, was 14% lower among KD mice, as compared to WT mice (Figure 2D). Exercise training had no effect on V_{max} or Hill coefficient in either KD+Ex or WT+Ex mice. Ca_{50}, which is defined as the concentration of free Ca^{2+} needed to activate SERCA to 50% V_{max}, did not differ between any of the experimental groups (Figure 2E).

Phospholamban content and phosphorylation was altered in KD mice

PLN mRNA was 52% lower in KD+Sed mice, as compared to WT+Sed mice (Figure 3A). Exercise training down-regulated PLN mRNA expression by 48% in WT+Ex mice and by 35% amongst KD+Ex mice as compared to their sedentary counterparts. KD+Ex mice also had 40% lower PLN mRNA, as compared to WT+Ex mice. PLN protein content was 23% lower in WT+Ex and KD+Ex mice as compared to their sedentary counterparts (Figure 3B). Since PLN inhibits SERCA2a protein function, we calculated the ratio of monomeric PLN:SERCA2a protein in each experimental group. The ratio of monomeric PLN:SERCA2a protein was 136% higher in KD mice, as compared to WT (Figure 3C). Exercise training reduced the ratio of monomeric PLN:SERCA2a protein amongst both WT+Ex and KD+Ex mice by 32%. Next, we assessed the phosphorylation status of PLN^{Thr17} (p-PLN^{Thr17}) and PLN^{Ser16} (p-PLN^{Ser16}) because unphosphorylated PLN inhibits SERCA2a at submaximal calcium concentrations (MacLennan et al. 1997). The phosphorylation of PLN^{Thr17} and PLN^{Ser16} sites occur via CaMKII and protein
kinase A (PKA), respectively (Simmerman et al. 1986). No difference in p-PLN<sub>Thr17</sub> was observed between WT+Sed and KD+Sed mice (Figure 3D). Exercise did not alter p-PLN<sub>Thr17</sub> protein levels in WT mice, but exercise training increased p-PLN<sub>Thr17</sub> by 61% amongst KD+Ex mice, as compared to KD+Sed mice. p-PLN<sub>Thr17</sub> protein levels were also 158% higher amongst KD+Ex mice, as compared to WT+Ex mice. The p-PLN<sub>Thr17</sub>:PLN ratio was significantly affected by exercise where the ratio was 62% higher amongst exercise trained mice compared to sedentary mice (Figure 3E). A different response was observed for p-PLN<sub>Ser16</sub>, where p-PLN<sub>Ser16</sub> was 49% lower amongst KD+Sed mice, as compared to their WT+Sed counterparts (Figure 3F). Although exercise training did not alter p-PLN<sub>Ser16</sub> phosphorylation in WT mice, exercise training did increase p-PLN<sub>Ser16</sub> phosphorylation by 194% in KD mice (P < 0.05), as compared to their sedentary counterparts. Even so, the relative levels of p-PLN<sub>Ser16</sub> phosphorylation was similar between WT+Ex and KD+Ex groups. A main effect of exercise training was identified for p-PLN<sub>Ser16</sub>:PLN ratio, where it was 130% higher amongst exercise trained mice, as compared to sedentary mice (Figure 3G).

Discussion

Our novel study is the first to explore the role of AMPK in regulating SERCA2a function, a major ATP utilization pathway in the myocardium, and suggests that AMPK influences energy homeostasis not only by regulating metabolic pathways that generate ATP, but also by regulating ATP utilization pathways. Exercise training increases SERCA2a protein levels in the hearts of mice (Kemi et al. 2007; Stolen et al. 2009) and rats (Epp et al. 2013; Tate et al. 1996), however, the specific mechanism by which this occurs remains unknown. The main finding of the current study is that exercise training increased SERCA2a protein content by nearly 50% in the hearts of
WT mice but had no effect on that of KD mice. These observations are the first indicating the importance of active AMPK in mediating exercise-induced effects on cardiac SERCA2a. Our data also extend the previous literature by reporting for the first time that SERCA2 mRNA, maximal SERCA activity, Hill coefficient and monomeric PLN:SERCA2a ratios are negatively regulated in left ventricles of KD mice, as compared to WT mice.

AMPKα influences the regulation of SERCA2 in the heart

Exercise training increased markers of metabolic and mitochondrial adaptation, namely total AMPKα, pACC:ACC and COX IV levels in WT and KD mice, however, p-AMPKαThr172 was increased only in WT mice. Other researchers have used a similar approach to characterize adaptations in skeletal muscle following exercise training (Cobley et al. 2012; Egan et al. 2013) and in the heart following hypoxia (Zungu et al. 2007). Our study found that exercise also increased the phosphorylation status of total p-AMPKαThr172 by 63% in the hearts of WT+Ex mice, as compared to WT+Sed mice. In contrast, p-AMPKαThr172 was not increased by exercise training in the hearts of KD mice that overexpress an inactivated AMPKα2 subunit. Coven et al. (Coven et al. 2003) previously reported that AMPKα2 activity in the rat heart is increased by 2.8- and 4.5-fold with moderate- and high-intensity exercise, respectively; while cardiac AMPKα1 activity was increased to a lesser extent at each intensity. Similarly, Nielsen et al. (Nielsen et al. 2003) have reported that the AMPKα2 activity and p-AMPKαThr172 levels increase, while AMPKα1 activity remains unchanged, in response to acute exercise at 80% of maximal aerobic capacity (VO2peak) in human skeletal muscle. Nielsen et al. (Nielsen et al. 2003) also reported that AMPKα2 protein content was not different, but that AMPKα1 protein content was higher, in skeletal muscle sampled from trained subjects compared to sedentary subjects. When comparing
KD to WT mice, we observed a 65% reduction in phosphorylated AMPKα Thr172 representing a net change in the phosphorylation status of both AMPKα-subunits as the antibody used recognizes both phosphorylated-AMPKα1 Thr172 and phosphorylated-AMPKα2 Thr172. Turdi et al. (Turdi et al. 2010) have previously reported that AMPKα2 activity was significantly reduced in KD mice, compared to age matched WT mice; whereas, AMPKα1 activity was unchanged by the overexpression of an inactivated AMPKα2 subunit. Habets et al. (Habets et al. 2009) determined that although the total protein levels of AMPKα1 and AMPKα2 were similar in cardiomyocytes isolated from of WT and KD mice, the activity of both isoforms in KD mice was impaired.

Our data add to the growing body of evidence indicating that exercise training increases myocardial SERCA2a levels (Epp et al. 2013; Kemi et al. 2007; Stolen et al. 2009; Tate et al. 1996) as exercise training increased SERCA2a protein content by 53% in WT mice. SERCA2 mRNA expression was not altered by exercise training in the current study and one possibility is a reduced rate of SERCA2a protein turnover. Kho et al. (Kho et al. 2011) have previously reported that the covalent addition of small ubiquitin-related modifier (SUMO1) to SERCA2a can prolong SERCA2a protein stability in the heart.

SERCA2a protein content was not altered by exercise training in the hearts of KD mice. In fact, KD+Ex mice had 60% lower myocardial SERCA2a protein content than their exercise trained WT counterparts, and was accompanied by lower SERCA2 mRNA levels. Since KD mice express an inactivated AMPKα2 subunit, it is possible that SERCA2 mRNA expression may be affected by signaling pathways that are affected by AMPKα signaling, such as sirtuin 1
AMPKα influences the regulation of PLN in the heart

PLN regulates intracellular calcium transport in cardiac tissue by directly binding to SERCA2a and, thereby, reducing the Ca\textsuperscript{2+}-sensitivity of the enzyme. Upon phosphorylation, PLN dissociates from SERCA2a, allowing increased myocardial Ca\textsuperscript{2+}-transport (Hovnanian 2007). In the current study, PLN protein levels were unchanged in KD mice, as compared to WT mice of the same training group as previously observed (Turdi et al. 2010). Coupled with lower SERCA2a protein levels, a 136% increase in the monomeric PLN:SERCA2a ratio was calculated in KD mice, as compared to WT mice. Increases in monomeric PLN:SERCA2a ratios have previously been reported in models of ischemia/reperfusion (Chohan et al. 2006), metabolic syndrome (Kagota et al. 2013), and type 1 diabetic heart (Vasanji et al. 2004) and would be expected to impair SERCA2 activity. Exercise training reduced total PLN protein levels in both WT and KD mice. This observation supports that of Stolen et al. (Stolen et al. 2009) in which exercise training reduces monomeric PLN:SERCA2 ratios. Although KD mice exhibited lower PLN mRNA levels compared to WT mice, PLN mRNA expression was significantly reduced by exercise in both KD and WT mice.

PLN can be phosphorylated at two sites (i.e. p-PLN\textsuperscript{Thr17} and p-PLN\textsuperscript{Ser16}). PLN\textsuperscript{Ser16} phosphorylation is regulated by β-adrenergic signaling via protein kinase A (PKA); whereas, PLN\textsuperscript{Thr17} phosphorylation is regulated by CaMKII (Simmerman et al. 1986). The data indicate that the level of p-PLN\textsuperscript{Thr17} in sedentary animals remained unchanged; whereas, the level of p-
PLN$^{\text{Ser16}}$ was lower amongst sedentary KD mice, as compared to their sedentary WT counterparts. Maarbjerg et al. did not observe any differences in activation of CaMKII in exercised KD mice (Maarbjerg et al. 2009), which may explain why p-PLN$^{\text{Thr17}}$ remained unchanged in sedentary animals in our study.

Exercise training did not alter p-PLN$^{\text{Thr17}}$ or p-PLN$^{\text{Ser16}}$ in WT mice. The data in the literature is mixed with one study showing exercise induced increases in p-PLN$^{\text{Ser16}}$ but no change in p-PLN$^{\text{Thr17}}$ content (Stolen et al. 2009) while another indicates exercise enhanced p-PLN$^{\text{Thr17}}$ and no change in p-PLN$^{\text{Ser16}}$ levels in WT mice (Kemi et al. 2007). Different exercise training models may have contributed to these discrepancies. In contrast to WT mice, exercise training increased p-PLN$^{\text{Thr17}}$ and p-PLN$^{\text{Ser16}}$ by 61% and 194%, respectively, in KD mice as compared to their sedentary counterparts. It is possible that this response amongst KD mice occurred as a strategy to maintain SERCA calcium-sensitivity, which would be supported by the lack of change in $\text{Ca}_{50}$ between experimental groups (Kemi et al. 2007; Stolen et al. 2009).

AMPK$\alpha_2$ KD mice are characterized by a prolonged cardiac deceleration time

Echocardiography was used to monitor changes in cardiac structure and function between sedentary and exercise trained WT and KD mice. Notably, LVID$_d$ was 13% larger in sedentary KD mice as compared to sedentary WT mice. These data differ from others in which mice ranging from 4-6 or 24-28 months of age, showed no changes in LVID$_d$ between WT and KD mice (Guo and Ren 2012; Turdi et al. 2010). In the current study, mice were 7 months old at the completion of the protocol; therefore, we cannot rule out the possibility that age-dependent changes may have contributed to observed difference (Turdi et al. 2010). Echocardiographic data
also indicate that LVID_d increased by 13% following exercise training in WT mice, as compared to sedentary WT mice. This observed cardiac adaptation to exercise is similar to that seen in endurance-trained athletes (Pluim et al. 2000); however, other studies have reported that exercise training does not alter LVID_d in humans who run at 70% of heart rate reserve for up to 50 minutes/day, 5 days/week for an average of 7 months (Wolfe et al. 1985). Likewise, mixed results have been reported in mice that perform sprint interval training 5 days/week for 8 weeks depending on their genotype (Riggs et al. 2010).

Diastolic function was also assessed in the current study. Notably, DT was prolonged by 43% in sedentary KD mice, as compared to sedentary WT mice. Exercise training enhanced the DT by 38% in KD mice so that the absolute values were similar to those observed for WT+Sed and WT+Ex mice. Sedentary KD mice may have impaired diastolic function that exercise training normalized to levels similar to those observed amongst WT mice. The observed improvement in DT was unexpected because exercise training did not increase SERCA2a protein levels or SERCA2 V_max in KD mice. Previous research has reported that SERCA2a function is correlated with contractility in the healthy and failing heart (Munch et al. 2000). However, we did not detect changes in LVEF or E/A ratio in the current study even though SERCA2a protein levels differed between KD and WT mice. Additionally, caution must be utilized when interpreting the change in DT because it is preload dependent. It is possible that the observed changes in PLN phosphorylation following exercise training in KD mice contributed to the faster DT time reported for this group in the absence of changes in SERCA2a protein content.

Limitations
The transgenic expression of the dominant kinase dead AMPKα2 protein isoform in the KD mice was restricted to cardiac and skeletal muscle using an MCK promoter. A strength of this KD mouse model is that our observations were not influenced by changes in AMPK expression in the kidney, pancreas, lung, spleen, brain, liver and adipose tissue (Mu et al. 2001). We report changes of protein expression due to exercise training and, therefore, must acknowledge that training status was different in WT and KD mice, as indicated by the lower running speed of KD mice for the graded exercise test. Golden et al. (Golden et al. 1994) have previously reported that exercise training did not induce a change in MCK expression in Wistar-Kyoto rats, but does alter MCK expression in borderline hypertensive rats or spontaneously hypertensive rats. We did not measure MCK expression in the current study, but also did not detect a difference for total AMPKα protein content between any experimental groups. Additionally, although the sample size used was powered to detect differences between groups, it was not powered to detect differences between sexes. Although we assumed Ca²⁺ handling is unchanged between male and female mice, there is recent evidence to the contrary in a different mouse model (Parks et al. 2014) and humans (Fischer et al. 2016); however, it remains unknown if cardiac SERCA2a levels are different between sexes in mice. Other Ca²⁺-handling proteins such as Na⁺/Ca²⁺ exchanger (NCX) may also contribute to the observed relaxation. NCX removes Ca²⁺ from the cell, whereas SERCA removes Ca²⁺ from the sarcoplasmic reticulum (SR), and these two proteins are reported to be involved in the reduction of SR Ca²⁺ levels in heart failure (Reviewed in Houser et al. 2000). In fact, a model of heart failure has proposed that a progressive reduction in the activity of SERCA could cause NCX activity to be increased as a compensatory mechanism (Hobai and O'Rourke 2001; Li et al. 2012). The current study is one of the first to explore the role of AMPK in regulating SERCA2a following stress induced by exercise-training.
however, further research examining changes in the regulation of Ca\textsuperscript{2+} such as Ca\textsuperscript{2+} transients, cardiomyocyte cell length during contraction, as well as changes in NCX, are warranted.

**Summary**

Collectively, these data suggest that an AMPK\textalpha-dependent mechanism influences the exercise-stimulated changes in SERCA2a protein content in the hearts of mice. This data adds to the literature by indicating that AMPK can be targeted by a physiological intervention (i.e. exercise training) to upregulate SERCA2a in the heart. Future work in this field should identify the specific processes by which AMPK regulates SERCA2a and PLN protein content.

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Conflict of Interest: No conflict of interests exist.
References


641 Mu, J., Brozinick, J.T., Valladares, O., Bucan, M. and Birnbaum, M. J. 2001. A role for AMP-
642 activated protein kinase in contraction- and hypoxia-regulated glucose transport in skeletal
644
646 SERCA2a activity correlates with the force-frequency relationship in human myocardium.
648
650 AMP-activated protein kinase (AMPK) is activated in muscle of subjects with type 2 diabetes
652
654 2003. 5'-AMP-activated protein kinase activity and subunit expression in exercise-trained
656
658 Ca(2+) release in murine ventricular myocytes are regulated by the cAMP/PKA pathway.
660
662
665
666 Richter, EA. and Ruderman, N. B. 2009. AMPK and the biochemistry of exercise: implications
668
670 interval training on the left ventricular morphology and function of VLCAD-deficient mice.
672
674 Skeletal muscle adaptation to exercise training: AMP-activated protein kinase mediates
675 muscle fiber type shift. Diabete, 56: 2062-2069.
676
678 activated protein kinase mediates ischemic glucose uptake and prevents postischemic cardiac
680
682 performance and SERCA by exercise and adiponectin gene therapy in insulin-resistant rat.
684
686 Reduced Ca(2+)-sensitivity of SERCA 2a in failing human myocardium due to reduced serin-


Figure captions

Fig. 1. LV protein expression of AMPKα, p-AMPKα\textsuperscript{Thr172}, p-AMPKα\textsuperscript{Thr172}:AMPKα ratio, ACC, p-ACC, p-ACC:ACC ratio and COX IV. (A) AMPKα. (B) p-AMPKα\textsuperscript{Thr172}. (C) p-AMPKα\textsuperscript{Thr172}:AMPKα ratio. (D) ACC. (E) p-ACC. (F) p-ACC:ACC ratio. (G) COX IV. †, main effect of genotype was observed for p-AMPKα\textsuperscript{Thr172}:AMPKα ratio and p-ACC where WT > KD (P < 0.05). Data are reported as mean ± SE. Two-way ANOVA was utilized followed by a Newman-Keuls post-hoc test. ‡, main effect of exercise was observed for AMPKα, p-ACC:ACC ratio, and COX IV, where Sed < Ex (P < 0.05). *, different from Sed of the same genotype (P < 0.05). #, different from WT from same training condition (P < 0.05). WT+Sed, wild-type mice, sedentary group; KD+Sed, AMPKα\textsubscript{2} kinase dead mice, sedentary group; WT+Ex, wild-type mice, exercise trained group; KD+Ex, AMPKα\textsubscript{2} kinase dead mice, exercise trained group.

Fig. 2. LV expression of SERCA2a mRNA, SERCA2a protein and calcium-dependent SERCA activity. (A) SERCA2a mRNA. (B) SERCA2a protein. (C) Maximal SERCA activity (V\textsubscript{max}). (D) Hill coefficient (η\textsubscript{H}). (E) Calcium sensitivity (Ca\textsubscript{50}). Data are reported as mean ± SE. †, main effect of genotype was observed for SERCA2a mRNA, V\textsubscript{max} and Hill coefficient, where WT > KD (P < 0.05). Two-way ANOVA was utilized followed by a Newman-Keuls post-hoc test. *, different from Sed of same genotype (P < 0.05). #, different from WT from same training condition (P < 0.05). WT+Sed, wild-type mice, sedentary group; KD+Sed, AMPKα\textsubscript{2} kinase dead mice, sedentary group; WT+Ex, wild-type mice, exercise trained group; KD+Ex, AMPKα\textsubscript{2} kinase dead mice, exercise trained group.
**Fig. 3.** LV expression of PLN mRNA and of proteins PLN, PLN:SERCA2a ratio, p-PLN<sup>Thr17</sup>, p-PLN<sup>Thr17</sup>:PLN ratio, p-PLN<sup>Ser16</sup>, p-PLN<sup>Ser16</sup>:PLN ratio. (A) PLN mRNA. (B) PLN. (C) PLN:SERCA2a ratio. (D) p-PLN<sup>Thr17</sup>. (E) p-PLN<sup>Thr17</sup>:PLN ratio. (F) p-PLN<sup>Ser16</sup>. (G) p-PLN<sup>Ser16</sup>:PLN ratio. Data are reported as mean ± SE. †, main effect of genotype was observed for PLN and PLN:SERCA2a ratio, where WT < KD (P < 0.05). ‡, main effect of exercise was observed for PLN, PLN:SERCA2a ratio, p-PLN<sup>Thr17</sup>:PLN ratio, and p-PLN<sup>Ser16</sup>:PLN ratio, where Sed > Ex (P < 0.05). Two-way ANOVA was utilized followed by a Newman-Keuls post-hoc test. *, different from Sed of the same genotype (P < 0.05). #, different from WT from same training condition (P < 0.05). WT+Sed, wild-type mice, sedentary group; KD+Sed, AMPKα<sub>2</sub> kinase dead mice, sedentary group; WT+Ex, wild-type mice, exercise trained group; KD+Ex, AMPKα<sub>2</sub> kinase dead mice, exercise trained group.
Table 1. Cardiovascular imaging parameters at 5 months.

<table>
<thead>
<tr>
<th></th>
<th>WT+Sed</th>
<th>KD+Sed</th>
<th>WT+Ex</th>
<th>KD+Ex</th>
<th>Main Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left ventricular end-diastolic diameter (LVIDd; mm)</td>
<td>2.4 ± 0.04</td>
<td>2.7 ± 0.05*</td>
<td>2.7 ± 0.09*</td>
<td>2.7 ± 0.05</td>
<td>-</td>
</tr>
<tr>
<td>Left ventricular posterior wall dimension (LVPWd; mm)</td>
<td>0.9 ± 0.01</td>
<td>0.9 ± 0.02</td>
<td>0.9 ± 0.02</td>
<td>0.9 ± 0.02</td>
<td>-</td>
</tr>
<tr>
<td>Ejection fraction (LVEF; %)</td>
<td>82 ± 1</td>
<td>79 ± 1</td>
<td>82 ± 2</td>
<td>82 ± 1</td>
<td>-</td>
</tr>
<tr>
<td>Endocardial velocity (cm/sec)</td>
<td>1.7 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>-</td>
</tr>
<tr>
<td>Early ventricular filling velocity (E wave; mm/sec)</td>
<td>613 ± 47</td>
<td>664 ± 45</td>
<td>651 ± 48</td>
<td>574 ± 29</td>
<td>-</td>
</tr>
<tr>
<td>Atrial ventricular filling velocity (A wave; mm/sec)</td>
<td>387 ± 42</td>
<td>379 ± 49</td>
<td>434 ± 47</td>
<td>356 ± 45</td>
<td>-</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>1.5 ± 0.08</td>
<td>1.7 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>1.8 ± 0.2</td>
<td>-</td>
</tr>
<tr>
<td>Deceleration time (DT; ms)</td>
<td>24.4 ± 1.8</td>
<td>34.9 ± 3.2*</td>
<td>26.9 ± 3.3</td>
<td>21.6 ± 1.1*</td>
<td>-</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>686 ± 11</td>
<td>701 ± 12</td>
<td>659 ± 16</td>
<td>658 ± 18</td>
<td>Main effect of exercise Sed&gt;Ex</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 8 mice per group). WT+Sed, wild-type mice housed in standard cages (sedentary condition). KD+Sed, AMPKα2 kinase dead mice housed in standard cages (sedentary condition). WT+Ex, wild-type mice housed in cages with voluntary exercise wheels (exercised condition). KD+Ex, AMPKα2 kinase dead mice housed in cages with voluntary exercise wheels (exercised condition). A main effect of exercise-training was observed for heart rate, where Sed > Ex. (P < 0.05). *, different from Sed of same genotype (P < 0.05). #, different from WT from same training condition (P < 0.05). Two-way ANOVA was utilized followed by a Newman-Keuls post-hoc test.
190x254mm (300 x 300 DPI)